Proceedings of the Joint Meeting AGI-SIBV-SIGA
Assisi, Italy – 19/22 September, 2011
ISBN **978-88-904570-2-9**

Poster Communication Abstract – 2A.69

WHEAT GENOTYPING BY ILLUMINA GOLDENGATE ASSAY: A TEST CASE

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SNP, Illumina GoldenGate assay, genotyping, wheat

Single nucleotide polymorphisms (SNPs) are efficiently used for building high resolution genetic maps, studying population evolutionary history and performing genome-wide association mapping experiments. These applications usually require genotyping of thousands of SNPs in a large number of individuals. Although a number of SNP genotyping assays are available, most of them are designed for SNP genotyping in diploid individuals. Genotyping of polyploid organisms is naturally complicated by the presence in the nucleus of homeologous and paralogous genes. In fact, SNP scoring in polyploid species might result in more than 3 clusters (AA, BB, AB) making SNP clustering sometimes more difficult and less accurate.

In this work, we used the Illumina GoldenGate assay for the medium-high throughput SNP genotyping of tetraploid and hexaploid wheat accessions. This technical approach allows to carry out the assay reactions directly on genomic DNA without a preliminary PCR amplification. Afterwards, the allelic state at an SNP locus is obtained using a custom oligo pool (OPA). These oligos are designed on the non-varying sequences around each SNP for 48 up to 384 SNP simultaneously, thus resulting cost, time and work effective and easily scaling up.

In particular, we used a 384-plex OPA (169 A-genome, 166 B-genome and 49 D-genome SNPs) for the screening of 420 polyploid wheat accessions including 210 lines of *T. durum*, 94 lines of *T. aestivum*, 116 lines of *T. dicoccoides*, and also 25 emmer lines (*T. dicoccum*) and three diploid species related to wheat, *T. urartu* (A genome), *A. speltoides* (B genome) and *A. tauschii* (D genome). Clustering of Cy3 and Cy5 normalized intensities in a Cartesian plot was used to infer the SNP genotypes.

The evaluation of these 384 SNPs allowed us to identify an initial SNP core-set that includes 191 SNPs generally scorable in any wheat material. Moreover, evaluating separately the different wheat sub-groups, the useful SNP number changed, as expected, in relation with the homology and genome polyploidy analyzed.

This study reinforces the idea that the GoldenGate assay could be a very efficient tool for high-throughput genotyping of polyploid wheat, opening new possibilities for the analysis of genetic variation in wheat and dissection of genetic basis of complex traits using association mapping approach.